

# INCIDENCE OF *TREPONEMA PALLIDUM* IMMOBILIZING ANTIBODY IN RABBIT IMMUNE SERA AGAINST VARIOUS TYPES OF MICRO-ORGANISMS AND VIRUSES\*

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The purpose of this paper is to report the results of systematic investigations into the serological relationship between *Treponema pallidum* and other micro-organisms and viruses. These investigations were particularly designed to discover whether the *T. pallidum* immobilizing antibody occurs in the sera of animals immunized with various bacteria and viruses. The data on the serological relationship between *T. pallidum* and other groups of micro-organisms are very scanty. The main handicap to the study of these problems in the past has been the lack of suitable methods for detecting true antibodies against *T. pallidum* *in vitro*. The *T. pallidum* immobilization test (TPI) was first described by Nelson and Mayer (1949). This test was an application *in vitro* of the demonstration of protective antibodies in sera from syphilitic rabbits *in vivo* (Turner, 1939). According to reports at present available, the TPI test should be a highly specific reaction for treponematoses, as no convincing example of false positive reaction with the TPI test has been demonstrated (Nielsen and Reyn, 1956). *T. pallidum* immobilizing antibody in immune sera was observed by Nelson and Diesendruck (1951) only in sera from rabbits infected with *T. pallidum*, *T. pertenue*, and *T. cuniculi*, whereas the treponemes were not immobilized by sera from rabbits injected with:

- (1) non-pathogenic spirochaete strains: Reiter, Kazan, and S-26;
- (2) *Leptospira icterohaemorrhagicae*, Strain, R-9040;
- (3) *Borrelia novyi*.

In 130 volunteers infected with *Plasmodium vivax*, Olansky, Harris, and Hill (1953) found that during infection with these parasites no significant increase of immobilizing antibody occurs. The lack of more

detailed studies on this subject was the reason for the present investigations (Krag, 1957).

## Material and Methods

The virulent Nichols strain of *T. pallidum* was used throughout these experiments. The sera examined were obtained chiefly from rabbits after immunization with living or more often with killed micro-organisms and viruses—killed by formalin or heat.† The level of antibodies for homologous antigen was usually high when determined by an appropriate test, e.g. agglutination, precipitation, and complement-fixation tests. The sera, with added merthiolate 1:10,000 in some cases, were kept in the refrigerator.

The TPI test was carried out exactly as performed at the Statens Seruminstitut in Copenhagen (Nielsen, 1957). Several controls were included:

(1) Each serum was separately incubated with a 40 per cent. (vol./vol.) of active and inactive complement (heated 30 min. at 56°C.); the inactive complement acts as a control of the survival of the treponemes in serum.

(2) A known positive serum was always included to ensure that the treponemes were able to react sufficiently with that serum, i.e. to control that the sensitivity on each experimental day was not too much different from that on any other day. In fact, the reactivity with the WHO Control Serum No. III in the experimental period was

$$\text{TPI} \frac{25(50)}{18-50/40}$$

400–600‡ in Copenhagen and 400–700 in Wroclaw.

† We are very much obliged to all who have furnished us with sera.

‡ The expression  $\text{TPI} \frac{25(50)}{18-50/40}$  is a special case of the general expression  $\text{TPI} \frac{d(e)}{a-b/c}$ , where *a* indicates hours of incubation, *b* the percentage of immobilization used for determining the end-point of the titration (in practically all cases 50 per cent.), *c* the volume percentage of complement used, and *d* the number of treponemes counted from each tube; *e* indicates that sometimes another number is counted, e.g. when the ratio living/dead is about 50/50.

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(3) A known negative serum was also tested to secure the survival of the treponemes in a mixture of this serum and active and inactive complement.

The results were read after 18 hrs' incubation at 35°C. Altogether 25 treponemes were counted and the numbers of mobile and immobile treponemes were noted. The specific immobilization was calculated as follows:

$$100 \times \frac{\text{per cent. mobile with inactive complement} - \text{per cent. mobile with active complement}}{\text{per cent. mobile with inactive complement}}$$

The results were interpreted as negative if the specific immobilization was less than 20 per cent.

After each tube had been read, 0.1 ml. 2.5 per cent.

( $5 \times 10^8$  cells/ml.) sheep cells in phosphate buffer (sensitized by three units 100 per cent. haemolysin) were added to all tubes to ensure the presence of residual complement activity in tubes to which active complement had been added, and to ensure that there was no complement activity in the other tubes. If the haemolysis was less than 100 per cent. the result for the immobilization test was considered unsatisfactory.

## Results

All results are listed in the Table, together with a more detailed description of the sera examined. As expected, the TPI results were non-reactive in each serum.

TABLE  
SERA EXAMINED

Organism and Reference	More Detailed Designation	No. of Sera Examined
Actinomyces (Kwapinski, 1958)	<i>A. israeli</i> (Strains 636, 649, 656, 661)	4
Mycobacterium (Wilson and Miles, 1955)	<i>M. tuberculosis</i> (1) B.C.G. strain (2) Human pathogenic strain (3) Tuberculoprotein	3
Corynebacterium (Poulsen, 1944)	(1) <i>C. diphth.</i> (Type gravis, 6 strains) (2) <i>C. diphth.</i> (Type mitis, 2 strains) (3) Unidentified, isolated from human being (3 strains) (4) <i>C. equi</i> (2 strains)	13
Streptococcus (Wilson and Miles, 1955)	<i>Strept. haemolyticus</i> (Group A, Types 1-30)	30
Escherichia coli (Ewing, Tatum, Davis, and Reavis, 1956)	(1) Test strains for O-antigens, 1-137 (2) Test strains for K-antigens, 1-79 (3) Test strains for H-antigens, 1-40	256
Alkalescens-Dispar (Kauffmann, 1954)	Test strains for O-antigens 1-8	8
Shigella (Kauffmann, 1954)	(1) <i>S. dysenteriae</i> (Types 1-8) (2) <i>S. flexneri</i> (Types: 1a, 1b, 2a, 2b, 3, 4a, 4b, 5, 6, variants X and Y) (3) <i>S. boydii</i> (Types 1-11 and provisional included serotypes: 703, M, 7a 2770/51, 425, Ca/792) (4) <i>S. sonnei</i> (S+R form)	37
Salmonella (Kauffmann, 1954)	(1) Group A: 1, 2, 12: a ( <i>S. paratyphi</i> A) (2) Group B: 1, 4, 5, 12: b ( <i>S. paratyphi</i> B) 4, 12: - ( <i>S. reading</i> - O serum) 1, 4, 5, 12: i-1, 2 ( <i>S. typhi</i> murium) 4, 5, 12: r-1, 2 ( <i>S. heidelberg</i> ) (3) Group C: 6, 7: c-1, 5 ( <i>S. cholerae</i> suis) 6, 8: e, h-1, 2 ( <i>S. newport</i> ) (4) Group D: 9, 12, Vi: d ( <i>S. typhi</i> ) 9, 12: g, q ( <i>S. moscow</i> ) 1, 9, 12: - ( <i>S. gallinarum</i> ) (5) Group E: 3, 10: - ( <i>S. london</i> - O serum) 3, 15: - ( <i>S. newington</i> - O serum) 3/1, 15/1, 34: - ( <i>S. illinois</i> - O serum) (6) Group F: 11: i-1, 2 ( <i>S. aberdeen</i> ) (7) Group G: 13, 22: z-1, 6 ( <i>S. poona</i> ) (8) Group H: 6, 14, 24: d-1, 5 ( <i>S. heves</i> ) (9) Group I: 16: b-e, n, x ( <i>S. hvingtoss</i> )	17
Klebsiella (Kauffmann, 1954)	Test strains for O-antigens 1-5 Test strains for K-antigens 1-7,	72
Haemophilus Bordetella	<i>H. influenzae</i> (Type b) (1) <i>B. pertussis</i> (4 strains) (2) <i>B. bronchiseptica</i> (13 strains)	18

contin.

Organism and Reference	More Detailed Designation	No. of Sera Examined
Leptospira (Wolff and Broom, 1954)	(1) <i>L. saxkoebing</i> (2) <i>L. mini</i> (3) <i>L. ballum</i> (4) <i>L. andamana</i> (5) <i>L. naami</i> (6) <i>L. djasimani</i> (7) <i>L. sarmini</i> (8) <i>L. sentoti</i> (9) <i>L. australis</i> (a) (10) <i>L. hardjo</i> (11) <i>L. cynopteri</i> (12) <i>L. benjamini</i> (13) <i>L. bangkinang</i> (14) <i>L. hyos</i> (15) <i>L. icterohaemorrhagiae</i> / AB/ (16) <i>L. canicola</i> (17) <i>L. bataviae</i> (18) <i>L. pyrogenes</i> (19) <i>L. hebdomadis</i> (20) <i>L. medanensis</i> (21) <i>L. autumnalis</i> (22) <i>L. australis</i> (b) (23) <i>L. semaranga</i> (24) <i>L. wolffii</i> (25) <i>L. sejroe</i> (26) <i>L. poi</i> (27) <i>L. javanica</i> (28) <i>L. pomona</i> (29) <i>L. grippotyphosa</i> (30) <i>L. paidjan</i>	30
Coxsackie Viruses (N.F.I.P., 1957)	(1) Group A, Types 1-19 (2) Group B, Types 1-5	24
Echo Viruses (N.F.I.P., 1957)	Types 1-14	14
Polio Viruses (N.F.I.P., 1957)	Types I-III	3
Influenza Viruses	Type A (Denmark) 57 Type A (Singapore) 57	2
Trypanosoma	(1) <i>T. rhodesiense</i> (2) <i>T. congolense</i> (3) <i>T. lewisi</i> (4) <i>T. cruzi</i> (5) <i>Crithidia fasciculata</i>	1 1 1 1 2
Leishmania	(1) <i>L. mexicana</i> (2) <i>L. donovania</i>	1 1
Total No. of Sera Examined		539

### Discussion

These results are not surprising. The TPI test has been performed in various countries and regions for several years, and many thousands of sera have been examined, but no convincing case of false positivity has been reported. The present investigations ought to have appeared some years ago, before the widespread employment of the TPI test.

The sera examined showed no content of immobilizing antibody and the conclusion must be reached that the organisms and viruses used in these immunizations have no antigens common with that of *T. pallidum* or (more cautiously) that these organisms have no antigen capable of provoking the production of treponemal immobilizing antibody.

The examined immune sera represent only a small

number of known human pathogenic micro-organisms and viruses; they were chosen because they are very common causes of infectious diseases, at any rate in the temperate zone, and were readily available at the Statens Seruminstitut or at the Ludwik Hirszfeld Institute of Immunology.

No special effort was made to examine immune sera from micro-organisms which are generally thought to be related to the treponemes, because (as Turner and Hollander, 1957, wrote): "There is no conclusive evidence indicating a close biological relationship between the treponemes and either the free-living spirochaeta, *Saprosira* and *Cristispira*, on the one hand or the more dependent genera such as *Borrelia* and *Leptospira* on the other hand. Indeed, there is much bacteriological, immunological, and

pathological evidence which suggests that the treponemes are no more closely related to these other genera of spiral organisms than to the many micro-organisms that are spherical or rod-shaped".

The employment of the TPI test alone in the present investigation means of course that it is especially interesting to demonstrate that this test is specific, *i.e.* that it is reactive only in cases of syphilis or other treponematoses. Had other methods also been used (*e.g.* the *T. pallidum* complement-fixation test (TPCF), the *T. pallidum* immune-adherence test (TPIA), the *T. pallidum* agglutination test (TPA), and perhaps tests using lipoid antigens) then reactivity might have occurred with some of the examined immune sera. Reactivity in some of these tests might have arisen from physico-chemical effects and not by antigen-antibody complexes alone. A more likely explanation is that the so-called non-specificity of, for example, the TPA test could depend on a common partial-antigen between the treponemes and other micro-organisms. Because of the controls in each case, reactivity in the TPI test is more probably an antigen-antibody reaction. In a complement-fixation test we can demonstrate only that some complement has disappeared, but in the TPI test we can demonstrate that the treponemes have been immobilized, and our controls tell us that this immobilization is a result of complement-activity together with another factor, namely antibody.

It is true that the present studies tell us only that the examined immune sera do not contain immobilizing antibody and that this answers only part of the question of the serological relationship between *T. pallidum* and other micro-organisms, but this answer is a very practical one.

The whole question can only be solved by investigations employing many methods including the reverse of that used in the present series, *i.e.* sera containing immobilizing antibody or more general antibodies against *T. pallidum* must be examined by various serological methods using various antigens treated in different ways from various micro-organisms.

### Summary

539 immune sera against various micro-organisms and viruses including various serological types of the genera and species selected were examined for immobilizing antibody against *T. pallidum* (Nichols strain) in the *T. pallidum* immobilization test. Immobilizing antibody was not demonstrated in any of these sera.

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